CLAIMS

1.   A method of determining the relative abundance of a plurality of proteins in a test sample compared to a reference sample, the method comprising:

(a)   providing a reference sample comprising a plurality of labelled proteins;

(b)   incubating a plurality of tagged antibodies capable of binding components of the reference sample with (i) a mixture of the labelled reference sample and the test sample and (ii) the reference sample alone, under conditions suitable for the binding of said antibodies to their targets;

(c)   comparing the amount of labelled protein bound to individual antibody tags in the presence and absence of the test sample.

2.   A method according to claim 1 wherein said test sample and reference sample are mixed in equal volumes.

3.   A method according to claim 1 or 2 wherein said antibodies are tagged with aluminium bar codes or dye impregnated beads

4.   A method according to any one of the preceding claims wherein each tag is linked to a single antibody species.

5.   A method according to any one of claims 1 to 3 wherein each tag is linked to more than one species of antibody.

6.   A method according to claim 5 wherein each of said antibody species linked to a tag binds the same protein.

7.   A method according to any one of claims 1 to 5 wherein each of said plurality of tagged antibodies binds a different protein.

8.   A method according to any one of the preceding claims wherein from 10^{11} to 10^{15} antibody molecules are bound to each tag.

9.   A method according to any one of the preceding claims wherein said reference sample is obtained from the same tissue and/or organism as said test sample.

10.   A method according to any one of the preceding claims wherein said reference sample is formed by pooling a plurality of test samples.

11.   A method according to any one of the preceding claims wherein said proteins in the reference sample are labelled with one or more fluorescent dyes.

12.   A method according to any one of the preceding claims wherein said binding is quantified by flow cytometry.

13.   A mixture of peptides wherein each peptide is of length n amino acids and of the formula:

$$X_1\text{-}X_2\text{-}X_3\text{-}...\text{-}X_n$$
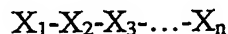
wherein:
-       each X represents an amino acid independently selected from one of a number of groups of amino acids;
-       each group of amino acids consists of less than 20 different amino acids,
-       n is the same for all peptides present in the mixture;
-       all of the following amino acids are present in at least one group: arginine, lysine, histidine, glutamate, aspartate, proline, cysteine, serine, threonine, tryptophan, glycine, alanine, valine, leucine, isoleucine, methionine, asparagine, phenylalanine, tyrosine and glutamine, and
-       for each peptide in the mixture the amino acid at the same position is selected from the same group.

14.   A mixture of peptides according to claim 13 wherein no amino acid is present in more than one of said groups of amino acids and/or each group of amino acids contains the same number of different amino acids.

15.   A mixture of peptides according to claim 14 wherein each X represents an amino acid independently selected from four groups of five amino acids or from two groups of ten amino acids and wherein no amino acid is present in more than one group.

16.   A mixture of peptides according to any one of claims 13 to 15 wherein each X represents an amino acid independently selected from one of two groups defined as follows:

(i)   arginine, lysine, histidine, glutamate, aspartate, proline, cysteine, serine, threonine, tryptophan;

(ii)   glycine, alanine, valine, leucine, isoleucine, methionine, asparagine, phenylalanine, tyrosine, glutamine.

17.   A mixture of peptides according to any one of claims 13 to 16 wherein n is 8.

18.   A library comprising a plurality of mixtures as defined in any one of claims 13 to 17 wherein each of said mixtures has the same value for n and the same groups of amino acids apply to all mixtures in the library, wherein (a) no peptide is present in more than one of said mixtures, and/or (b) the mixtures differ by virtue of the fact that the combination of groups chosen to obtain the peptides differs between the mixtures and optionally the library comprises mixtures representing all possible combinations of the groups.

19.   A library according to claim 18 wherein each of said mixtures comprises a different tag.

20.   A library according to claim 18 or 19 wherein said library comprises all possible peptides of length n.

21.   A library according to any one of claims 18 to 20 wherein the groups of amino acids are defined as follows:

(i)   arginine, lysine, histidine, glutamate, aspartate, proline, cysteine, serine, threonine, tryptophan;

(ii)   glycine, alanine, valine, leucine, isoleucine, methionine, asparagine, phenylalanine, tyrosine, glutamine.

22.   A method of detecting a plurality of immunoglobulins in a test sample, the method comprising:

(a)   providing a plurality of tagged antigens;

(b)   incubating said tagged antigens of (a) with said test sample, under conditions suitable for the binding of any immunoglobulins present in said test sample to their targets;

(c)   incubating said mixture of (b) with one or more labelled antibodies capable of binding specifically to immunoglobulins;

(d)   measuring the amount of labelled antibody bound to each tagged antigen.

23.   A method according to claim 22 wherein said plurality of antigens comprises oligopeptides and/or oligosaccharides.

24.   A method according to claim 22 or 23 wherein each of said antigens comprises a different tag.

25.   A method of any one of claims 22 to 24 wherein said antigens are sub-divided into mixtures, each mixture comprising a different tag.

26.   A method according to claim 25 wherein said antigens are peptides divided into mixtures on the basis of their amino acid sequence.

27.   A method according to claim 26 wherein said mixtures are as defined in any one of claims 13 to 17.

5   28.   A method according to claim 26 wherein said plurality of antigens is a library as defined in any one of claims 18 to 21.

29.   A method according to any one of claims 22 to 28 wherein said labelled antibodies comprise antibodies specific to two or more immunoglobulin

10   subclasses.

30.   A method according to claim 29 wherein said antibodies specific to each immunoglobulin subclass comprise a different label.

15   31.   A method according to claim 29 or 30 wherein said immunoglobulin subclasses are selected from IgG1, IgG2, IgG3, IgA, IgD, IgE and IgM.

32.   A method according to any one of claims 22 to 31 further comprising the step of quantifying the amount of each immunoglobulin subclass that binds each

20   tagged antigen or tagged antigen mixture.

33.   A method according to any one of claims 22 to 32 wherein the amount of labelled antibody bound to each tagged antigen or tagged antigen mixture is measured by flow cytometry.

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34.   A method of detecting the presence of, or a susceptibility to, a disease or other medical condition comprising:

(v)   detecting a plurality of immunoglobulins in a test sample obtained from an individual; and

30   (vi)   comparing the immunoglobulins detected in the sample from said individual with known patterns of immunoglobulins associated with the presence or

absence of a disease and thus determining whether said individual has, or is susceptible to said disease.

5   35.   A method according to claim 34 wherein said patterns of immunoglobulins associated with disease are determined by a method comprising:

(i)   ˙ detecting a plurality of immunoglobulins in test samples obtained from individuals whose disease status is known;

(ii)   comparing the immunoglobulins detected between those individuals who are disease sufferers and those who are not and identifying any patterns

10   associated with the presence or absence of the disease;

36.   A method of detecting the presence of, or a susceptibility to, a disease or other medical condition comprising:

(i)   detecting a plurality of immunoglobulins in test samples obtained from

15   individuals whose disease status is known;

(ii)   comparing the immunoglobulins detected between those individuals who are disease sufferers and those who are not and identifying any patterns associated with the presence or absence of the disease;

(vii) detecting a plurality of immunoglobulins in a test sample obtained from an

20   individual by the same method used in part (i); and

(viii) comparing the immunoglobulins detected in the sample from said individual with the patterns identified in step (ii) and thus determining whether said individual has, or is susceptible to said disease.

25   37.   A method according to any one of claims 34 to 36 wherein said detecting is carried out by a method of any one of claims 22 to 33.

38.   A method according to any one of claims 34 to 37 wherein said comparing is carried out using a pattern recognition method selected from Principal

30   Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), genetic computing, a support vector machine, linear discriminant analysis, variable selection algorithms and wavelet decomposition.

39.   A method according to any one of claims 34 to 38 which aids the diagnosis of a disease, aids the prediction of a future disease, aids the assessment of the severity of a disease, aids the monitoring of progression or regression of a disease or aids the monitoring of treatment of a disease in said individual.

40.   A method according to any one of claims 34 to 39 wherein said disease is coronary heart disease.

41.   A kit suitable for use in a method of any one of claims 22 to 40 said kit comprising

(iii)   a plurality of antigens or mixtures of antigens, wherein each antigen or mixture of antigens comprises a tag; and

(iv)   one or more labelled antibodies capable of specifically binding to immunoglobulins.

42.   A kit according to claim 40 wherein said antigens are defined as in any one of claims 23 to 28

43.   A kit according to claim 41 or 42 wherein said labelled antibodies are defined as in any one of claims 29 to 31.

44.   A kit according to claim 41 comprising

(i)   a library of peptides as defined in claim 21 wherein each group of antigens is tagged with aluminium barcodes; and

(ii)   a labelled antibody capable of specifically detecting human IgG.

45.   A method of reducing the redundancy and bias of an antibody-expressing phage library comprising:

(a)   providing two surfaces to which a sample of antigens is bound wherein said antigens are bound to the second surface at a higher density than to the first surface;

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(b)   exposing a phage display library to a first surface of (a) under conditions suitable for antibody binding and selecting phage bound to said surface;

(c)   exposing said selected phage of (b) to a second surface of (a) under conditions suitable for antibody binding and selecting phage not bound to said surface;

(d)   optionally further selecting said phage of (c) according to steps (b) and (c) one or more times;

thereby obtaining a library of antibody-expressing phage which has reduced redundancy and/or bias characteristics compared with the original library.

46.   A method according to claim 1 wherein said plurality of antibodies is an antibody-expressing phage library produced according to the method of claim 45.